IN THE UNITED STATES PATENTS AND TRADEMARKS OFFICE

In re application of
H. SHIMIZU et al. : Examiner: PULLIAM, A
Serial No. 09/600,744 : Group Art Unit: 1615
Filed on July 20, 2000 :
For: PRODUCTION METHOD FOR SUSTAINED-RELEASE PREPARATION

DECLARATION UNDER 37 CFR 1.132

Honorable Commissioner of
Patents and Trademarks,
Washington, D.C. 20231

Sir:

I, Mr. Kei Mukai, a citizen of Japan, residing at 16-4, Kisaichi 2-chome, Katano-shi, Osaka, Japan sincerely declare:

That I was born on April 26, 1966 in Hirakata-shi, Osaka, Japan and graduated from the department of Chemical Engineering, Kyoto University, Japan in March, 1992;

That I have been employed by Takeda Chemical Industries, Ltd., Osaka, Japan since April, 1992, and have been engaged in research in pharmaceutics at the Pharmaceutical Development Laboratories of the Pharmaceutical Production Division since 1992;

That I am a member of the Chemical Engineering Society, Japan;

That I am a co-inventor of the above-identified U.S. Patent Application SN. 09/600,744; and

That I sincerely declare that, under my direction and/or control, the following experimentation was conducted:

Experiment

A. Method for producing microspheres

A1) Preparation of sustained-release MC (1-month preparation) [MC powder (1M); cf. Flow A1]

2.4g of gelatin and 15.2g of Leuprorelin acetate were dissolved in 15.0g of distilled water under warming. To this

solution, 321g of a separately prepared dichloromethane solution of a lactic acid-glycolic acid copolymer (hereinafter referred to as PLGA) [lactic acid/glycolic acid = 75/25 (mol%), weight-average molecular weight 10,500] (121g of PLGA contained) was added, followed by emulsification with stirring using a mini-mixer for 2 minutes (rotation rate 10,000 rpm). This emulsion was added to 25L of a previously prepared 0.1% aqueous solution of polyvinyl alcohol (PVA), followed by emulsification again. While this W/O/W emulsion was gently stirred, the solvent was removed over a period of about 3 hours. The MCs obtained were passed through a 75μm sieve to remove coarse particles, then centrifuged. The MCs separated were washed with distilled water to remove the free drug and PVA, after which they were subjected to wet sieving through sieves of 90μm pore size in the presence of a small amount of distilled water. 18.4g of D-Mannitol was added to the product suspension and dissolved to yield a MC suspension. Then the suspension was lyophilized and sieved to yield MC powder.

A2) Preparation of sustained-release MC (Placebo) [MC powder (Placebo); cf. Flow A2]

Previously prepared 25L of 0.1% aqueous solution of polyvinyl alcohol (PVA). To this solution, 320g of a separately prepared dichloromethane solution of a lactic acid-glycolic acid copolymer (hereinafter referred to as PLGA) [lactic acid/glycolic acid = 75/25 (mol%), weight-average molecular weight 10,500] (121g of PLGA contained) was added, followed by emulsification. While this O/W emulsion was gently stirred, the solvent was removed over a period of about 3 hours. The MCs obtained were passed through a 75μm sieve to remove coarse particles, then centrifuged. The MCs separated were washed with distilled water to remove PVA, after which they were subjected to wet sieving through sieves of 90μm pore size in the presence of a small amount of distilled water. 18g of D-Mannitol was added to the product suspension and dissolved to yield a MC suspension. Then the suspension was lyophilized and sieved to yield MC powder.

B. Comparative Examples [cf. Scheme 1 and Picture 1~3]

B1) Experimental Example 1 - Mannitol

An ice layer with thickness of about 2mm was previously formed on a tray for lyophilizing (a width of 170mm, a length of 270mm and a depth of 45mm) at about -40°C using water for injection. The ice layer was also formed on the inner wall of the tray (ice-lining). 33g of Mannitol was dissolved in 500mL of water for injection with cooling at about 5°C. The solution was added on the tray on which the ice layer had been formed. After thoroughly freezing at about -40°C, the sample was lyophilized by the conventional method.

An observation of the lyophilized sample after lyophilization revealed that the sample had been scattered.

B2) Experimental Example 2 - MC powder(Placebo)

An ice layer with thickness of about 2mm was previously formed on a tray for lyophilizing (a width of 170mm, a length of 270mm and a depth of 45mm) at about -40°C using water for injection. The ice layer was also formed on the inner wall of the tray (ice-lining). 52g of MC powder(Placebo), in which no active ingredient was encapsulated, was dispersed in 200mL of water for injection with cooling at about 5°C. The suspension was added on the tray on which the ice layer had been formed. After thoroughly freezing at about -40°C, the sample was lyophilized by the conventional method.

An observation of the lyophilized sample after lyophilization revealed that no scattering had occurred.

B3) Experimental Example 3 - MC powder(1M)

An ice layer with thickness of about 2mm was previously formed on a tray for lyophilizing (a width of 170mm, a length of 270mm and a depth of 45mm) at about -40°C using water for injection. The ice layer was also formed on the inner wall of the tray (ice-lining). 52g of MC powder (1M), in which Leuprorelin Acetate was encapsulated, was dispersed in 200mL of water for injection with cooling at about 5°C. The suspension was added on the tray on which the ice layer had

been formed. After thoroughly freezing at about -40°C, the sample was lyophilized by the conventional method.

An observation of the lyophilized sample after lyophilization revealed that no scattering had occurred.

The comparative results and photographs clearly show that when the production method is applied for microsphere suspension, there is no undesirable scattering of the preparation. In contrast, if the method is run using aqueous solution dissolving hydrophilic compound or chemical entity, such as mannitol, quite a large amount of scattering of the preparation is evident.

The comparative results and photographs also show that this result is independent of the ingredients contained in the microsphere, as the same desirable outcome is obtained when the active ingredient leuprorelin acetate is contained in the microsphere as when no active ingredient is contained in the microsphere.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

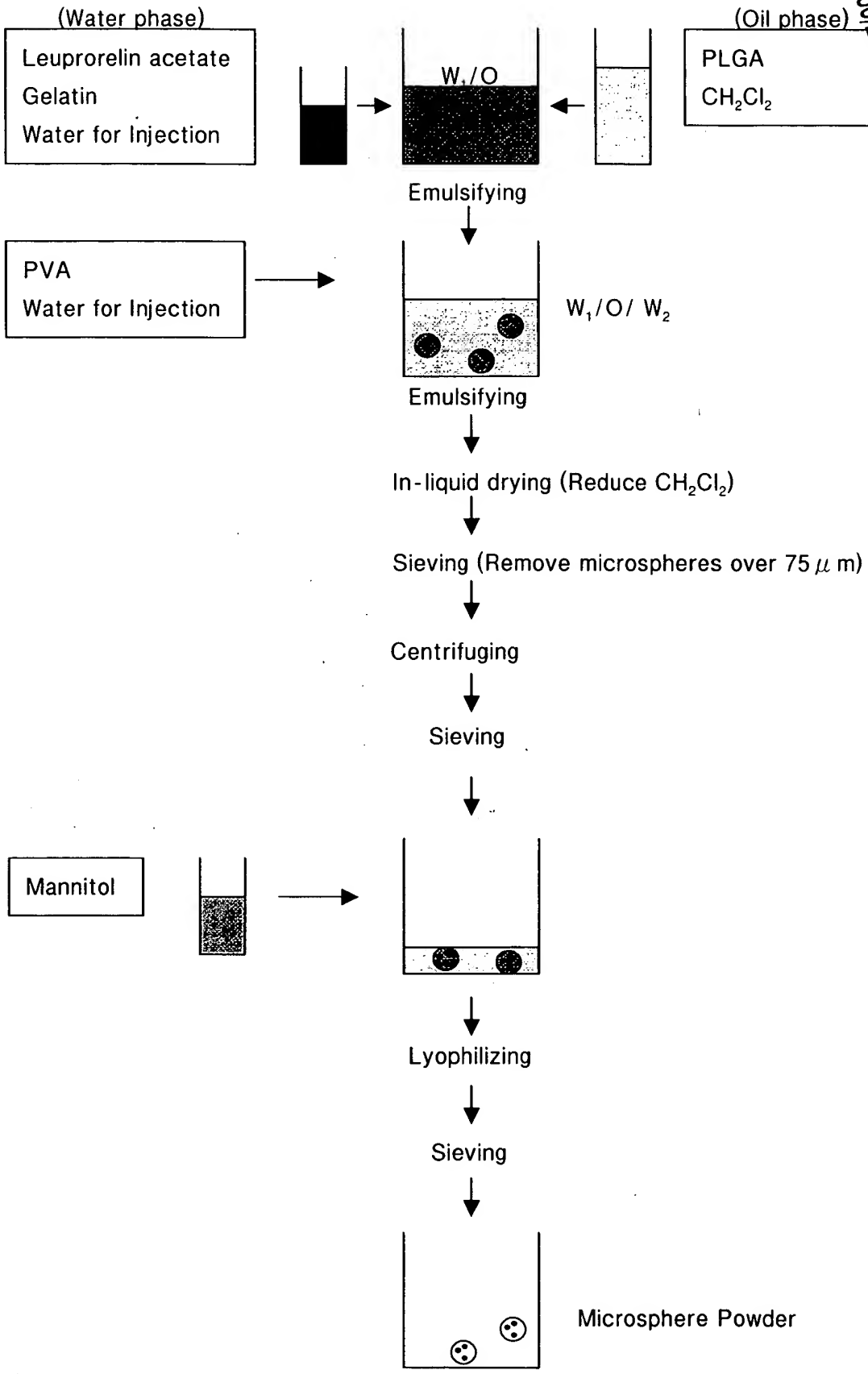
Signed at Osaka, Japan this 14th day of February, 2003

Kei Mukai

Kei Mukai

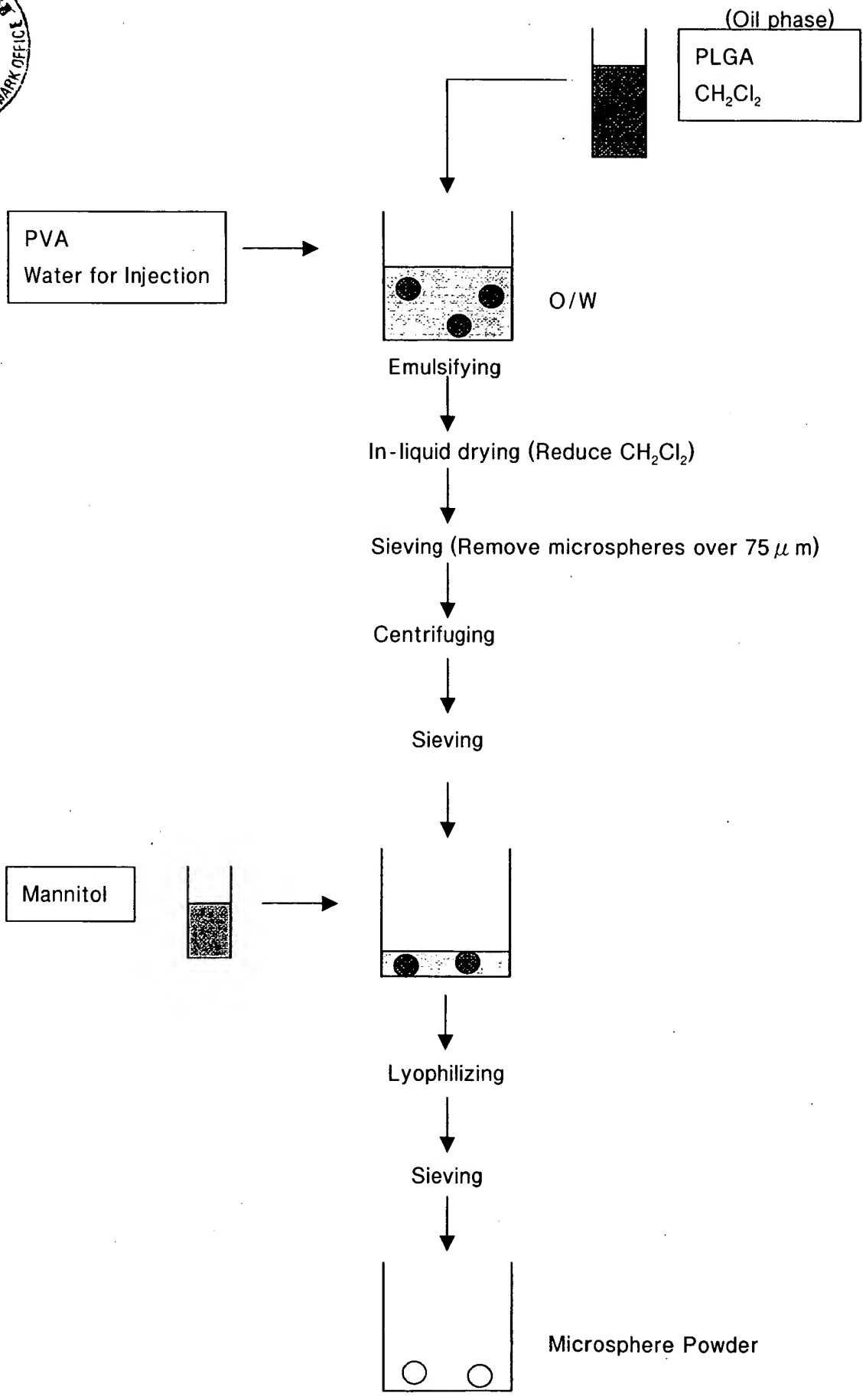
Flow A1

Schematic Flow of the Manufacturing (TAP-144-SR)



Flow A2

Schematic Flow of the Manufacturing (TAP-144-SR Placebo)



Picture 2

4. Feeding the Solution and the Slurry

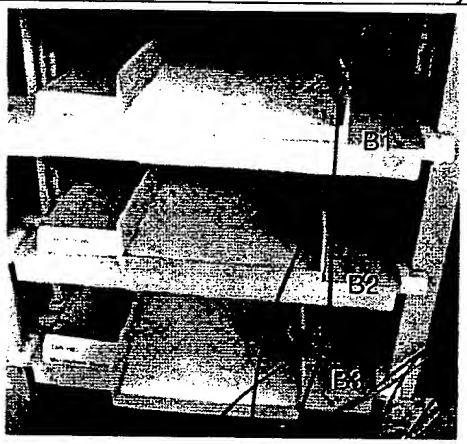


Fig.. 4

Each the solution of Mannitol and the slurry of MC powders was added on the trays for lyophilizing on which the ice layer had been formed respectively. After thoroughly freezing at about -40℃, the samples were lyophilized by the conventional method.

upper shelf : Mannitol [B1]
middle shelf : MC powder (Placebo) [B2]
lower shelf : MC powder (1M) [B3]

5. After lyophilization

Fig.. 5

Lyophilized cake of Mannitol had been scattered. An observation of the lyophilized samples of MC powder (Placebo) and MC powder (1M) revealed that no scattering had occurred.

upper shelf : Mannitol
middle shelf : MC powder (Placebo)
lower shelf : MC powder (1M)

Picture 1

Comparative experiments of ice-lining

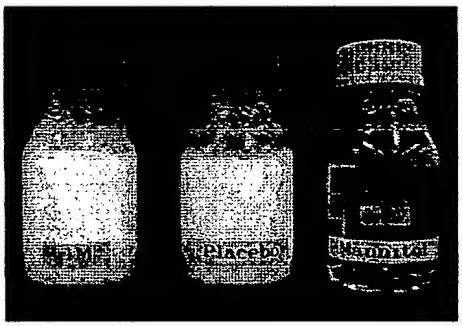
1. Weighing

B3 B2 B1

Fig.. 1

Mannitol :33g
MC powder(Placebo) :52g
MC powder(1M) :52g
Checkweighed respectively(Fig.1)

2. Dissolution/Suspension



B3 B2 B1

Fig.. 2

Mannitol was dissolved in 200mL of water for injection.

MC powder (Placebo) and MC powder (1M) were dispersed in 200mL of water for injection.

3. Ice-lining

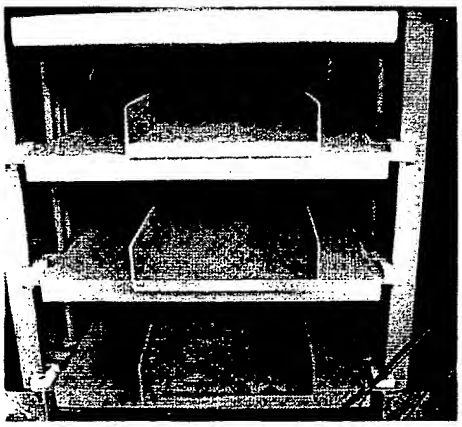
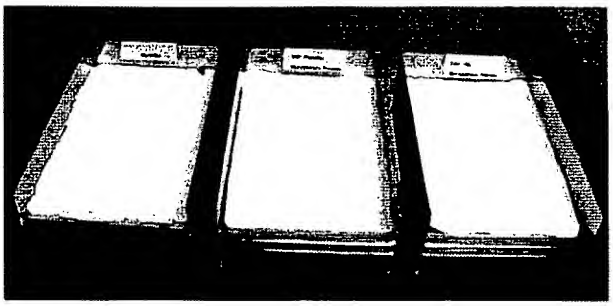


Fig.. 3

An ice layer with a thickness of about 2mm was formed on the bottom and the inner wall of the tray at about -40 ℃ using water for injection. (Ice-lining)

Picture 3

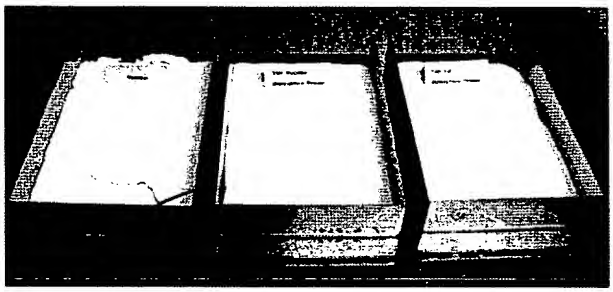
6. The appearance after lyophilization



B1 B2 B3

Fig.. 6 (Forward)

Left tray : Mannitol [B1]
Center tray : MC powder (Placebo) [B2]
Right tray : MC powder (1M) [B3]



B1 B2 B3

Fig.. 7 (Backward)

Left tray : Mannitol
Center tray : MC powder (Placebo)
Right tray : MC powder (1M)

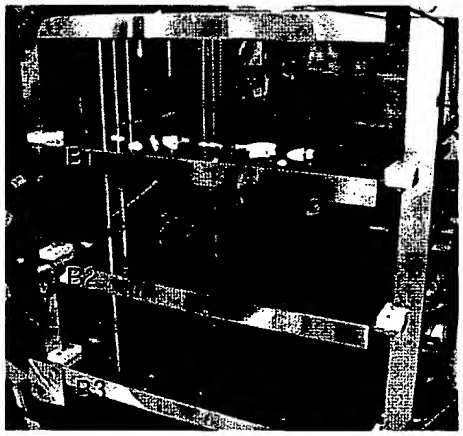


Fig.. 8

Inside appearance of a lyophilizer after removing the trays

Confirmed scattering Mannitol cake

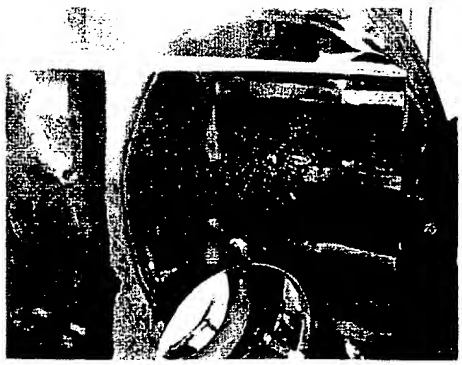


Fig.. 9

Internal side of the door of a lyophilizer

Mannitol had been scattered at the upper part of the door.

Scheme 1

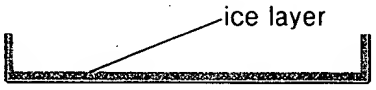
2501USOP :「ice-liner freeze-dry method」 a scheme of comparative experiment

① preparation of a tray for freeze-drying

a tray for freeze-drying (W 170mm、L 270mm、H 45mm)

② ice-lining

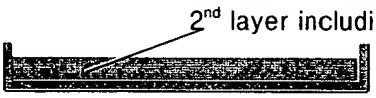
Water for injection is poured and sprayed on a tray (container) under low temperature (ex. –40℃). So, an ice-coating layer (1st layer) is prepared on the tray.



ice-lining(ca. 2mm thick)

③ preparation of second layer

Next, a mannitol solution or a suspension of microspheres is added to the 1st layer and then the solution and the suspension are frozen. Consequently, two layers are prepared.



poring a solution of an object of freeze-dry be cooled to 5℃

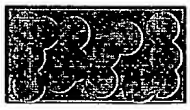
(1)Mannitol *solution*(D-mannitol 33g／200mL water for injection)

(2)Microsphere *suspension*(MC powder (1M) 52g／200mL water for injection)

(3)Microsphere *suspension*(MC powder (Placebo) 52g／200mL water for injection)

※ comparing 2nd layer

mannitol solution being homogenized (=dissolved)



microsphere suspension being made of heterogeneous layer in which particles (=microspheres) are dispersed.

④ after freeze-dried

Then, these double layers are freeze-dried. Water of the 1st layer and water of the frozen-mannitol-solution or water of the frozen-microspheres-suspension (2nd layer) are removed.

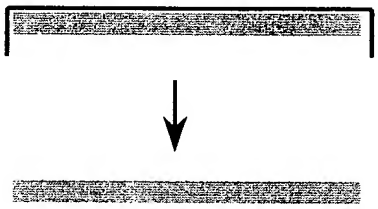
(a)Mannitol :Representation of hydrophilic chemical entities

(b)Microspheres (1M)

(c)Microspheres (Placebo)

Finally, only freeze-dried microspheres are prepared on the tray.

⑤ Recover of the object



Then, since the portion of the 1st layer is empty and there is a space between the tray and the freeze-dried microspheres in a form of sheet or film are recovering from the tray only by inverting the tray and tapping the bottom of tray without scraping freeze-dried microspheres.

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